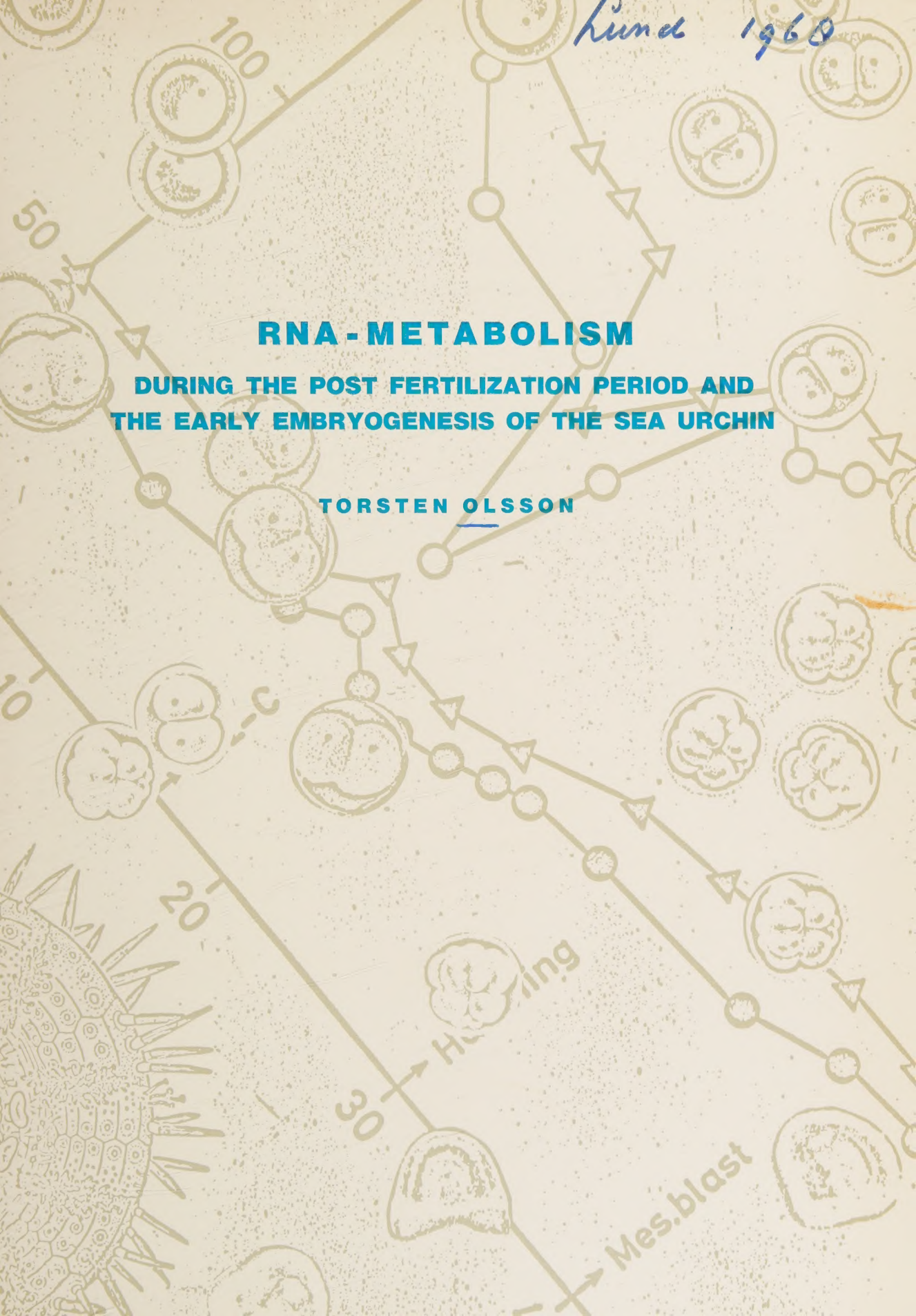


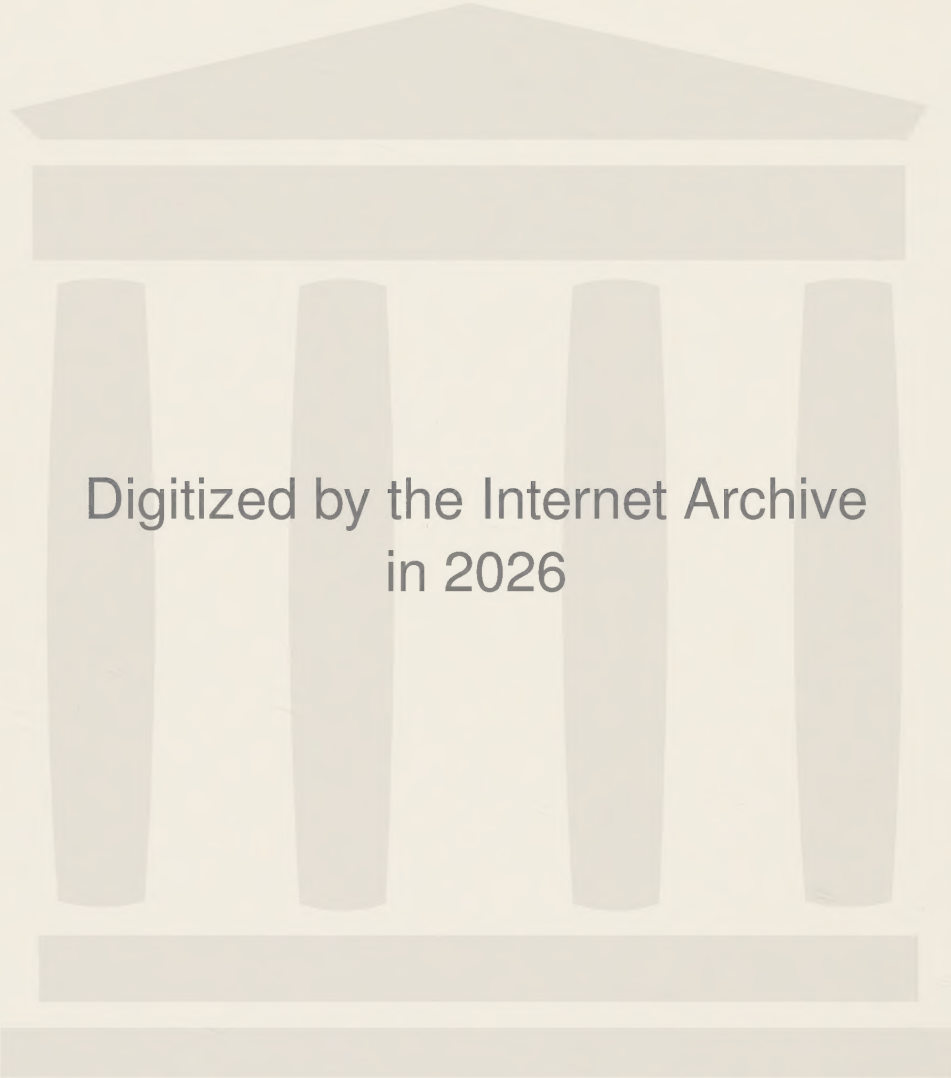
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RNA - METABOLISM

DURING THE POST FERTILIZATION PERIOD AND
THE EARLY EMBRYOGENESIS OF THE SEA URCHIN

TORSTEN OLSSON





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Ribonucleic Acid Metabolism during the Post Fertilization
Period and the Early Embryogenesis of the Sea Urchin

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RIBONUCLEIC ACID METABOLISM DURING THE POST FERTILIZATION
PERIOD AND EARLY EMBRYOGENESIS OF THE SEA URCHIN

BY

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FIL. LIC. LD

By due permission of the Faculty of Science
of the University of Lund

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for the degree of Doctor of Philosophy

The present dissertation is based on results partly from investigations concerning fertilization physiology (I, II, III) and partly from studies related to developmental physiology or chemical embryology (IV, V) in the field of nucleic acid. The mentioned investigations are compared with related studies by other authors and are discussed from a current point of view.

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The mentioned papers will be referred to by the Roman numerals.

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A. Nucleic Acid - an Analytical Comment

A complete hydrolysis of a nucleic acid (NA) yields purine and pyrimidine bases, sugar components and phosphoric acid. A partial hydrolysis of the NA structure gives nucleotides and nucleosides. The latter consist of purines or pyrimidines condensed with ribose or deoxyribose and the nucleotides are the phosphoric esters of the nucleosides.

If the pentose included is deoxyribose, the NA formed is deoxyribonucleic acid (DNA) and if the sugar is ribose the NA is named ribonucleic acid (RNA). The genetic information is carried mainly by the DNA present in the chromosomal structure. The information is transferred to RNA in order to make this NA capable of guiding the protein synthesis in the cell.

The RNA appears in different degrees of polymerization and brings about different functions in the synthesis. Usually the RNA is classified in the three groups presented below. For the analysis of the NA in the works presented (I-V) acid extraction and hydrolysis have been used besides extraction with phenol. Analysis by acid treatment principally according to methods described by Schneider (1945) and by Ogur and Rosen (1950) have been used in (II) and (IV) respectively, i. a., in order to correlate the present investigation with earlier studies.

The phenolic extraction method, modified from Kirby (1956) for sea urchin material, has been regarded as the basic method in the analyzing of RNA. The method has been especially adapted to make possible a selective estimation of ribosomal RNA in a homogenate from eggs or embryos.

1. Ribosomal RNA (r-RNA)

The RNA appears in the ribosomes in the cytoplasm. In sea urchin material the sedimentation of the r-RNA after phenolic treatment is usually

registered in the sections 18 and 28S. Its molecular weight is about $1-2 \times 10^6$. In accordance with the adaptation of the phenolic method the r-RNA is obtained in the aqueous phase (Aq-RNA) after the extraction (Slater and Spiegelman, 1966, Giudice and Mutolo, 1967).

2. Messenger RNA-(m-RNA)

This type of RNA supplies genetic information to the ribosomal structure with which they form polysomal aggregates. It has a lower degree of polymerism than the r-RNA and the sedimentation, depending on the isolation method used, is about 8-16S. The molecular weight usually does not reach 10^6 . The m-RNA that partakes in the formation of monosomes might be included to some extent in the Aq-RNA fraction. In the way in which the phenolic extraction is performed, all the nuclear RNA will be found in the protein-bound fraction in the phenolic phase. This RNA (Ph-RNA) will thus include the nuclear m-RNA and the nuclear store of ribosomal structures. The appearance of extranuclear RNA with specific affinity for proteins may also be possible in the Ph-RNA (Singer and Leder, 1966).

3. Transfer RNA (t-RNA)

The adding of amino acids to the polypeptide chains according to the code given by the m-RNA is mediated by the t-RNA. This RNA is often included in a group called soluble RNA (s-RNA). The sedimentation is around 4S and the molecular weight ranges about 2×10^4 .

It is difficult to trace this cytoplasmic RNA type with certainty in the fractions from the phenolic extraction and there has been no serious intention in the investigations (I-V) to determine s-RNA.

B. Introduction

The fundamental functions assigned to RNA in the protein synthesis of the cell are well-known facts. Before it is morphologically possible to trace a differentiation of an embryonic structure, a marked increase in the amount of RNA is noted in the actual part of the embryo. Generally speaking, different types of RNA mediate the genetic information and induce and administrate a regional synthesis of structural and catalytic proteins resulting in an organized embryonic structure.

In order to follow the change of the RNA-pattern during the early embryogenesis, a continuous analysis of whole embryos during the first stage of the development is of great importance as a complement to cytochemical and autoradiographic studies or analysis of subcellular structures. The value of a "whole embryo analysis" of higher differentiated developmental stages may be questionable, but an analysis of stages up to, for example, the blastula gives interpretable results with appropriate techniques. The experimental design in the present investigations was further dictated from the presumption of an existence of a rapid fluctuation in the NA metabolism in fertilized eggs and on the whole in early developmental stages. Earlier investigators in the field have not been able to catch the details from the fast moving pictures because samples for analysis were usually taken at too wide intervals. Less adequate analytic methods have also been used in many works. In biochemical studies of embryologic material unrevised "cook-book-formulas" are largely inapplicable. The author has paid much attention to an adaptation of the techniques to the material, resulting, among other things, in the use of a phenolic extraction method in the RNA analysis.

The choice of the research animal employed in the study is an important question. At first it may appear most suitable to choose an animal species as high ranking as possible in the animal kingdom in order to have the "egoistical" allusions to Homo as distinct as possible. However, in order to

get a reliable biochemical analysis of early development it is necessary to have a large amount of eggs and early embryos, all at the same and well-defined developmental stages. It is impossible to use mammalia for the analyzing of, for example, the metabolism in the egg in immediate connection with fertilization. Further, the simplified picture of life in low-ranking animals can often give a more general idea of metabolic processes, and reproducible analytical data may be more easily obtained.

For the investigation summarized in this paper, the sea urchin, a marine animal in the Echinoid group, has been used. The animals hold an eminent position as research objects in investigations related to the above-mentioned field of physiology, and as a curious fact Hertwig's discovery in 1876 of the fertilization process was made in sea urchin material. The meiotic cleavage of the ripened eggs is completed and the fertilization is performed in sea water, outside the animal and directly initiate the start of the caryogamic period. This artificial insemination and the absence of the polar body formation give a high degree of natural developmental synchrony and millions of embryos can be included. Beside this phenomena the absence of yolk in the egg and the incapability of including metabolites from the surroundings make the material favorable for biochemical investigations. The absence of yolk not only favors the chemical analysis but also the microscopic analysis, thus providing an excellent possibility of correlating the structural developmental data with biochemical findings. In the investigations summarized two species of sea urchin have been used: *Paracentrotus lividus* from the Mediterranean Sea (Naples) and *Echinus esculentus* from the west coast of Sweden (Kristineberg). The species have been chosen in order to furnish a valuable comparison between the results obtained from animals with different developmental rates and originating from different milieus.

C. The RNA Metabolism during the One-Cell Stage

The reaction initiated by the acrosomal contact preceding the plasmogamy in fertilization is a primary phenomenon in the cytoplasmic activation of the egg. This structural change of the cytoplasm can be seen as a preparation for the caryogamy but, on the other hand, this is only one among many processes partaking in the syngamy. The complex reactions in the egg at fertilization and during the time until the first cleavage have been the subject of many review articles, among which may be mentioned Allen and Pasteels 1958 in the symposium The Chemical Basis of Development, Runnström et al 1950 in The Cell I, Brachet 1960 in The Biochemistry of Development and Monroy 1965 in The Biochemistry of Animal Development.

a. RNA and Fertilization

The change in permeability (Runnström, 1924. Hobson, 1932. Steward and Jacobs, 1932) is one of the well-known results of the activation of the sea urchin egg and it illustrates well the close interaction between the structure and the function (Öhman, 1945. Monroy and Montalenti, 1947. Epel, 1967). Some compounds pass inactively through the membrane according to their varying concentration; the passage of other substances, however, is dependent upon an active process connected with phosphorylation (Piatigorsky and Whiteley, 1965). Structural alterations as a result of stimulation of the cell seem to be a general phenomenon, and some authors have tried to find a resemblance between, for example, the reaction in the egg after the acrosomal contact and the reaction in the nerve cell after an electrical stimulation (Hiramoto, 1959. Tyler and Monroy, 1959. Hori, 1965).

The structural change in the sea urchin egg at fertilization might point to an alteration of an "ion-exchange capacity" of the cytoplasm rather than

to only a variation of a more peripheral "sieve effect" of the cortex (Mirsky, 1936. Monroy-Oddo and Esposito, 1951). The author, by introducing a plasmolysis test (unpublished), has also got an impression of rapid fluctuation not only in the cortical region but also in the internal cytoplasmic structure during the first 20-30 minutes after the fertilization. Hagström and Lönning (1961) have showed the result of the activation in a change in the content of nucleic acids and proteins around the nucleus a few minutes after fertilization. Furthermore, it may be mentioned that the author, by using electron microscopy, has noticed a cytoplasmic change around the nucleus some minutes after fertilization (unpublished). Obviously the cortical change seems to be an initial reaction that extends through the cytoplasm. It has been noted that the egg contains in its peripheral parts ribosomal structures (Lancing and Rosenthal, 1949. Endo, 1961) which become disintegrated during the spreading of the cortical reaction on the egg surface. These ribosomes of informosomes include besides NA also proteolytic enzymes and must be considered as rather important participants of a lysosomic character. A distribution of parts of essential m-RNA from the disintegrated NA-particles seems to be one of the primary steps in the activation and resulting in the formation of active polysomal aggregates (Mano and Nagano, 1966. Mano, 1966).

Comparison of analytical results from the one-cell stage and from other cleavage stages must be made very critically in order to eliminate sources of uncertainty. It may, however, be of some interest to mention a result from a study of cultured mammalian cells. In these cell populations the metaphase ribosomes lack the capacity of synthetizing proteins because of the temporary presence of a protein coat at the breakdown of the nucleus. The ribosomes do not function until the onset of the interphase stage. However, by treatment with proteolytic enzymes the isolated metaphase ribosomes in vitro were made to form proteins (Gross and Fry, 1965). The presence of proteolytic enzymes in the cortical NA-structure of the unfertilized egg could facilitate the addition of the m-RNA to the cytoplasmic ribosomes at the fertilization. These ribosomes possibly have a protein coating that must be disintegrated as a prerequisite to the polysomal formation and the onset of the protein synthesis (Hultin, 1961, 1964).

Monroy et al, 1965. Ficq, 1966). The NA structure of the cortical region seems to be the source of both the m-RNA and the proteolytic enzymes, and a possible direct interaction between these two components also has been assumed (Stavy and Gross, 1967).

The foregoing discussion is intended to give information about problems related to the presented analysis of the one-cell stage and to provide an appropriate background for a continued examination and ventilation of the results obtained by the author about the NA-metabolism (I) (II) (III). The most pronounced events during the first minutes after fertilization are the described cortical reaction and the formation of the fertilization membrane derived from the vitelline coat. By extracting the RNA with phenol for analysis the author noted a decrease in a high-polymeric fraction of ribosomal type (Aq-RNA) during the first minutes after fertilization, (I) (II). This result is especially pronounced for *Paracentrotus*, as can be seen in (II). From a later study Slater and Spiegelman (1966) have described a similar phenomenon in the sea urchin *Arbacia*. The drop in Aq-RNA is taken by the author as an indication of a decomposition of a ribosomal structure. In the same investigation a RNA (Ph-RNA) with a more pronounced affinity for protein revealed an increase during this initial part of the development. The increased Ph-RNA may be seen as a result of the same disintegration phenomenon. The NA-products from the decomposition of the ribosomal structure may, for example, exhibit a strong bonding to certain cell proteins and thus be obtainable in the Ph-fraction of NA.

In the species *Paracentrotus* an examination of the ribonuclease has also been made and an increase of the enzymatic activity related to unfertilized eggs has been noted during the first minutes after fertilization (I). The rise in the activity may fit well into the NA-picture presented. As stated by Lundblad and Hultin (1954), the fertilization results primarily in an activation of ribonuclease followed by an activation of proteolytic enzymes. Investigations have shown, as mentioned, the presence of both RNA and proteolytic enzymes in cortex, and in order to release the protein-splitting enzymes as high-polymeric RNA, perhaps functioning as a coat, may be disintegrated. Such a sequence of

events seems to be quite acceptable and is supported by the investigations presented in this paper. An increased amount of acid-soluble nucleotides (AN) would also be expected as a result of the increasing activity of ribonuclease. For *Paracentrotus* a slight rise in this fraction is noted but the reverse is seen for *Echinus* (II). However, in the work reported in (II) the material has been prepared as acetone powder and most of the nucleotides certainly have been extracted by the acetone and lost for analysis.

In order to trace variations in the amount of AN and also to try to determine changes in the egg structure, experiments including extraction of AN from intact unfertilized and fertilized eggs in PCA for varied lengths of time were performed (I) (III). Samples for analysis were taken from the breeding bath as far as possible every minute in order to follow the rapid fluctuations immediately after fertilization. Using an appropriate time of extraction it was possible to extract a conspicuously high amount of AN during the period limited to the cortical reaction. An outflow of nucleotides at this time is probably due to a breakdown of high-polymeric RNA-structures. In a comparison between the curves in (I) illustrating the extractability of AN and the activity of ribonuclease the liberated amount of AN seems to be in correlation with the enzymatic activity. The curves shows principally the same course but exhibit a separation in the time scale, and therefore the resemblance, however, must be interpreted with some reservations. The interpretation of extractability of the AN must also be made with caution because the cytoplasmic structure is subjected to pronounced changes during the period. The bonding conditions between proteins and nucleotides may be altered and result in a varied extractability independent of the amount liberated by the enzymes.

b. RNA and Aster Formation

The part of development discussed above includes principally phenomena appearing in a more immediate connection with the fertilization. The

following is intended to describe events occurring during the remaining part of the one-cell stage. Developmental periods involving obvious alterations in the fertilized egg, are the stages of spermaster and amphiaster formation. In the spermaster development an apparent increase in the cytoplasmic viscosity of the egg is described by Fry and Parks, (1934) and also registered in the plasmolysis test mentioned above.

The intension of the studies presented in (I) and (II) was to determine whether the formation of the aster is reflected in the continuous analysis of RNA during the course of the one-cell stage. Other investigators have shown that the mitotic apparatus maintains a relatively high amount of RNA (Zimmerman, 1960). Hultin (1961) has described the presence of RNA as essential for the synthesis of proteins necessary for the cleavage. By experiments including the use of labelled substances the contribution of RNA in aster formation has been confirmed. An increased incorporation in the protein skeleton of the aster points to a novo synthesis (Stafford and Iverson, 1964. Mangan et al, 1965). In other works, the synthesis seems to a lesser degree to concern structural parts because the label is associated mostly with enzymatic proteins (Wilt et al, 1967). Some investigations, on the other hand, speak about a reorganization of pre-existing proteins in the aster formation. From the results first present in report (I), the author, as mentioned, has tried to map out eventual variations in the RNA-profile and correlated the obtained data with observations of the asters formations. Preferably extraction with phenol has been used in the analysis (II), where a high-polymeric RNA-fraction (Aq-RNA) shows pronounced variations. A clear increase in the amount of this RNA is noted during the period of development where spermaster and amphiaster are visible in the egg. During the same stage, the protein-bound Ph-RNA shows decreasing values. From the experiments reported in (I) (II) the author concluded that the building of an aster structure requires the presence of a high-polymeric ribosomal type of RNA and possibly a temporary accumulation of polysomes. The co-operation of such a type of

RNA makes a protein synthesis very probable and, in spite of the difference in origin between the spermaster and amphiaster, the RNA-pattern, related to the method used, seems to be the same.

D. The RNA Metabolism during Early Embryogenesis

To a great extent the initiation of the protein synthesis in the sea urchin egg after the sperm entry is made possible by the fact that the instructions and plans (m-RNA) for the embryonic structure have already been delivered by the mother. The machinery (ribosomes) necessary for the production of the building material (protein) is also made and supplied by the mother. All that remains to be done in order to start building, is to unpack the machines. This "unpacking" is probably done, as mentioned, by the use of proteolytic enzymes. However, the maternal building instructions describe only the fundamental constructions. For a more functional elaboration of the building and for an arrangement of the technical installations, i. e., when the morphological and physiological differentiation of the embryo is to take place, the maternal guidance must be complemented. In addition to maternal instructions paternal direction is now also required. A new set of machines must also be made and a large part of the old set modified for the production of the more elaborate structures now required and outlined in new drawings (m-RNA). From the activation at fertilization until a stage where the renewals or reorganization occur, as mentioned, a maternal program is followed. The duration of this programmed period, compared to many other types of cells (Scott and Bell, 1964), lasts for a long time especially if the time of storage in the unfertilized eggs is included. The stage of reprogramming, morphologically best characterized as a preblastula stage, occurs for *Paracentrotus* 5 hours and for *Echinus* 15 hours after fertilization. During this maternal period the embryo shows a very stable metabolic state not easily influenced by chemical or physical treatment. The stability may be referred to the type of metabolic system guiding the development during this period, a system differing in many respects from the one in use after the preblastula stage.

a. The Existence of a NA-Complex

In the investigations presented the author has paid only superficial attention to the DNA. However, the existence of a cytoplasmic storage DNA or a NA-complex calls for a short discussion (IV). The presence of this NA-complex in eggs and early developmental stages in several species has been proposed by other authors and their investigations have been reviewed by Grant (1965). In the present study the existence of a NA-complex has been suggested from the result of the acid extraction (OR III) and also from the phenolic extraction (Ph-RNA) of the material (IV). The appearance of this NA-fraction in the acid extract only after treatment with hot PCA (OR III) indicates a stable bonding to cellular proteins and it may be quite possible to expect the NA-complex in the Ph-RNA after phenolic extraction. A chromatographic separation of the purines and pyrimidines of the mentioned Ph-RNA has demonstrated a changing relation between the purines during the development. The relation of guanine to adenine was high in eggs but showed decreasing values until the blastula stage where the relation was about 1,0. In sea urchins Agrell (1964), by using other methods, also has described a NA-fraction rich in guanylic acid and existing only during the cleavage.

It has been discussed in the literature whether the complex appears diffusely or particularly in the cytoplasm. The author has in (IV) proposed the possibility of an eventual identity between the whole or part of the complex fraction and the maternal messenger. A bonding to yolk platelets has been suggested by Baltus and Brachet, (1962) and a localization in mitochondria has been reported by other authors (Pikó and Tyler, 1965. Pikó et al, 1967). As the NA-complex seems to exist only during the developmental period until the preblastula stage, it is possible to assume a temporary existence of a certain fraction of specialized mitochondria including parts of the NA-complex. This fraction may then be replaced by new mitochondria perhaps including other enzymatic systems than the old ones. As a matter of fact, a change in the regional distribution of mitochondria in the embryo (Agrell, 1953) and also in the amount

(Gustafsson and Lenique, 1955) has been observed around the blastula stage. A quantitative and especially a qualitative change would contribute to an understanding of the general metabolic adjustment at the early blastula stage based on enzymes included in these subcellular particles and concerning, for example, respiration and fat metabolism. In an electron-microscopic examination, the author (unpublished) has found groups of mitochondria together with fat vacuoles in eggs and in successively decreasing amounts in embryos up to the preblastula stage, but never after this stage.

b. RNA-Metabolism during the Pre-Blastula Stage

An adjustment of the NA-system and consequently also of the protein-synthesizing system during the early embryogenesis has been described for different species and shows principally the same expression. The phenomenon appears at slightly different developmental stages in varied groups of animals, but it seems to be a general occurrence necessary for the further differentiation of the embryo (Crippa et al, 1967). Among important questions, the statement of an eventual breakdown and replacement of ribosomes at this critical stage has played an important role.

Parallel investigations but with different methods performed by Comb and Brown (1964) and by the author (Olsson, 1965) have shown a marked decrease in ribosomal RNA in sea urchin embryos around the preblastula stage. The registered mode of decrease differs between the two investigations but may be principally of the same nature. The change noted by the author (Olsson, 1965) (IV) is of short duration and appears about 5 hours after fertilization in *Paracentrotus* and 15 hours after fertilization in *Echinus*. The reprogramming and reactivation of ribosomal structures is a rapid event (Bachvarova et al, 1966) and the change registered by the author in the ribosomal fraction may perhaps be limited to only one or a few cellular cleavages where the resolved nucleus leaves programmed ribosomes or parts of ribosomes to the cytoplasm (Samarina

et al, 1967). Possibly also degraded parts of the maternal ribosomes move to the nucleus in order to receive for example, new m-RNA. In the actual investigations by the author and also by Com and Brown (1964) an increase in a nuclear RNA fraction is in fact registered during the stage of ribosomal degradation.

In view of the mentioned facts it may be postulated with great certainty that there is a profound change in the ribosomal arsenal in the cell at the pre-blastula stage.

Studies performed more recently have indicated differences between maternal ribosomes and ribosomes of later stages in sea urchins (Nemer and Infante, 1967). The conclusions are founded on results from centrifugation experiments. This gradient analysis has also given results pointing to an exchange from one polysomal structure to another around the blastula stage (Infante and Nemer, 1967). As the nucleus is of definite importance for the synthesis of new ribosome structures (Edström et al, 1961). Brown and Gurdon, 1964), the appearance of this organelle at the stage in question (Agrell, 1966) also support the assumption of a synthesis of new r-RNA.

In connection with the discussion on transcription and way of programming, the temporary sensibility to actinomycin at the preblastula stage described by the author (Olsson, 1965) (V) may be mentioned. The sensibility to the drug may point to a temporary RNA-polymeraseactive period at this stage. The disturbance of the development as a result of actinomycin treatment may depend primarily on an influence of the drug upon the transcribing of m-RNA from the chromosomal DNA. However, not only "structural genes" producing m-RNA may be disturbed but also the production of copies from "regulator genes" may be influenced. The synthesis of r-RNA in the nucleus has also been described as sensitive to actinomycin (Schwartz and Garofalo, 1967) and a deficit in the production of ribosomal particles may also be manifested as an inhibition of development.

Regarding the appearance of the nucleolus at the blastula period a contact between this organelle in formation and the drug may perhaps permanently spoil the ability of the nucleolus to synthesize m-RNA. The proposal in

(V) that an inhibition of a transcription of a fundamental code at the preblastula stage cannot be compensated in later developmental stages may also be regarded as plausible. Denis (1966) has demonstrated in amphibians the existence of a m-RNA population with varied turnover and coding possibilities and Silver and Comb (1967) have mentioned a temporary m-RNA synthesis in sea urchins reaching a maximum at early blastula.

Actinomycin may inhibit different mechanisms and the possibilities should be taken into consideration in an interpretation of the results. However, an important finding in this study is the fact that the sensitive period is restricted to only the preblastula region of the development. After the demonstration of the actinomycin sensitivity being limited not only to the "beginning" but also to "the end" (Olsson, 1965) other investigators have found the same phenomenon by using actinomycin treatment for not too long periods during the development (Barros et al, 1966).

c. RNA and Basic Proteins

This paper is not directly concerned with protein analysis but some questions related to basic proteins and their relation to different groups of RNA and their influence upon the embryonic development will be touched upon here. The ribosomes consist not only of RNA but also of basic proteins. A problem that has been discussed for some time is the source of the ribosomal protein. The question is not easy to answer and has a resemblance to the famous question about the hen and the egg! Today most authors designate already existing ribosomes as suppliers of this integrating constituent to new members (Sypherd, 1966). If it is possible for all ribosomes or for some specialized groups to deliver basic proteins, we may assume a possible delivery not only to other ribosomes but also to chromosome structures. In this way it may also be possible for ribosomes to interact with the transcription from the chromosomes by a selective synthesis of, for example, histones. The basic proteins or

histones are also found to have an influence upon the bonding of m-RNA to ribosomes and can, obviously, in this way guide the polysome formation (Lindsay, 1966). This behavior resembles in some way the action of interferon or interferon-induced proteins synthesized after viral infections (Marcus and Salb, 1966. Carter and Hilton, 1967). From the mentioned relationship basic proteins seem to interact in three ways with the protein synthesizing system. Firstly, the basic protein must be included in the ribosomes themselves. Secondly, the protein may interact in the transcribing by coating the chromosomes. This covering may be affected via an induction-repression mechanism, or via a direct hormonal action (Sluyser, 1966). Thirdly, the regulating effect upon polysomal structures may be reflected in the translating at the synthesis of cytoplasmic proteins. The effect manifested by histones upon development has been demonstrated by a direct administration of these basic proteins. In stages of the developmental period when the embryo requires a selective transcription from the chromosomes, histones have a pronounced inhibitory effect on the development (Brachet, 1964. Molinaro and Cusimano-Carollo, 1966).

Finally, in connection with this part of the discussion, a work by Silver and Comb (1967) and also an unpublished observation of the author may be mentioned. The former investigators have described the identification of an acetic acid soluble nuclear protein synthesized selectively during the early blastula stage in sea urchins. The unpublished observation is related to the phenolic extraction of *Echinus esculentus*. In some series the acid hydrolysis of the Ph-phase, including nuclear RNA, has been performed as a fractionated procedure including successive sampling for spectrophotometric examination during the course of the hydrolysis. At stages around the early blastula the RNA was found to be more easily hydrolyzed and extracted from the protein compared to stages before or after this developmental period also when the higher amount of RNA in the Ph-phase at the stage in question is taken in consideration. From this observation it may be concluded that nuclear RNA during this stage may be bound to a protein of a more basic character. These results may principally also be compared with observations and comments in a study by Agrell and Bergqvist (1967).

E. Conclusions

The principal results from the investigations presented in the paper (I) - (V) may be summarized as follows:

1. After adapting the phenolic extraction method to the embryonic material used, the method revealed a decrease in a high-polymeric RNA of ribosomal type during the first minutes following fertilization. The same type of RNA shows maximum values in developmental stages where the spermaster and amphiaster have pronounced extension. A low-polymeric RNA exhibit minimum values when the mentioned ribosomal type shows a maximum, (I), (II).
2. A method of fractionated acid extraction was introduced in analyzing the one-cell stage. A marked temporary flow of easily extractable nucleotides into the acid was noted during the development of the fertilization membrane. A second rise in the amount of the extractable nucleotides was seen at the stage of beginning spermaster formation, (I) (III).
3. A cyclic variation in the ribonuclease activity was registered immediately after fertilization, (I).
4. When analyzing the nucleic acids during embryogenesis, using the modified phenolic extraction, a marked temporary drop is noted for cytoplasmic RNA of ribosomal type in the preblastula stage. A rise in the

amount of a low-polymeric, protein(nucleus)-bound RNA is registered for the same developmental stage, (IV).

5. A successively decreasing amount of guanine related to adenine, in the protein-nucleus-bound RNA fraction from the phenolic extraction, is indicated for the stages between the fertilization and the blastula (IV).
6. The amount of acid-soluble nucleotides exhibits a decreasing tendency after the preblastula stage, (IV).
7. By using actinomycin treatment for consecutive short periods during the embryonic development a limited transcribing or reprogramming period has been indicated during the preblastula stage, (V).

F. Acknowledgement

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